Introduction

- Fetal alcohol spectrum disorder (FASD) is the outcome of prenatal alcohol exposure (PAE) that causes neurobehavioral disorder associated with a wide range of adverse effects that have an impact in the development of the embryo by reducing the capacity to adapt to stress and altering neuroimmune factors in the amygdala that results in cognitive, mood and neuroimmune disorders.
- PAE negatively impacts the brain stress-response system by dysregulating the expression of stress neuropeptides such as corticotropin releasing hormone (CRH), and neuroimmune factors including interlukin-1beta (IL-1β). CRH and IL-1β are suspected to play a role in chronic anxiety and depression.
- The increase in cortisol provides a negative feedback system to decrease the amount of CRH released from the hypothalamus. However, during chronic stress, hyperactivity of HPA axis and activation NLRP3 signaling in microglia may induce the pro-inflammatory cytokine, IL-1β, and increase levels of cortisol.
- In animal models, studies have characterized the key brain region, the amygdala, in regulating stress and emotion such as anxiety and depression.
- Prior research demonstrates that stress enhances amygdala activity and alters the expression of neuroimmune factors. However, it is not well understood whether PAE alters the expression of stress and neuroimmune factors in the amygdala in response to stress.

Hypothesis: PAE alters protein levels of CRH and IL-1β in the amygdala following an early life stress.

Methods

Animals: All procedures were approved by the University of New Mexico Health Sciences Center (UNMHSC) Institutional Animal Care and Use Committee and adhered to practices recommended in “Recognition and Alleviation of Pain and Distress in Laboratory Animals” (Committee on Pain and Distress in Laboratory Animals. Institute of Laboratory Animal Resources, National Institutes of Health, National Academy Press, Washington D.C. 2011). All animals were maintained on a reverse 12-hour light/12-hour dark schedule (lights off at 0800 hours). Postnatal day 10 male and female offspring (10 days of age) were used for the present studies.

Prenatal Alcohol exposure (PAE):

Maternal separation stress: Litter animals were randomly assigned to control or maternal separation. Postnatal day 10 (PND10) pups were subjected to a 4-hour maternal separation stress starting two hours into the dark cycle (10:00). The maternal separation pups were isolated from their mother and control littermates and placed together in a cage in a different room for 4 hours. Maternal separated pups had the same standard nesting material as the home cage and were placed on a similar vented rack. Control mice stayed in the home cage with their dams, undisturbed, for four hours.

Tissue Collection: Brain was removed and dissected into amygdala (AMG) and was snap-frozen in liquid nitrogen and stored at -80°C until use.

Protein Isolation: Treated with Qiazol, chloroform, guanidinium hydrochloride, cell lysis buffer 2 (R&D Systems).

Protein expression of CRH, IL-1β, FKBP5 and NLRP3 in mice amygdala were detected using enzyme-linked immunosorbent assay (ELISA) kits.

Transcription Factors FKBP5 and NLRP3 in PND10 male mice

Figure 1: Protein levels increased significantly in CRH (stress hormone) in male mice that were prenatal alcohol exposed. This trend is observed in pro-inflammatory cytokine, IL-1β.

Figure 2: Protein levels were similar for CRH and IL-1β in prenatally alcohol exposed female mice. There is no change in male mice that were given saccharin (control group).

Figure 3: A trend of increased in FKBP5 and NLRP3 in male mice that were prenatal alcohol exposed. There is no change in male mice that were given saccharin (control group).

Figure 4: Protein levels of FKBP5 and NLRP3 show no change in female mice that were prenatal alcohol exposed.

Stress hormone and Pro-inflammatory cytokine in PND10 male mice

Stress Hormone and Pro-inflammatory cytokine in PND10 female mice

Results

- CRH and IL-1β protein levels increased significantly in PAE male mice at PND10 in both unstressed and stressed groups. (PAE) interaction F(1,18) = 4.617, P=0.045
- A trend towards increased FKBP5 and NLRP3 protein levels were observed in unstressed and stressed PAE male mice compared
- Trends towards decreased CRH, IL-1β and FKBP5 protein levels were observed in PAE female mice in both regardless of exposure to stress.
- These results are suggestive of sex differences in the amygdala from PAE but not saccharin control mice. Elevated protein levels of CRH and IL-1β were observed in male offspring independent of stressor exposure.

Conclusions and Future Research

The results from this study show the amygdala from non-PAE mice integrates stress responses that are adaptive and supports a return to normal brain levels of hormone and inflammatory factors, while these factors in the amygdala from PAE mice remain elevated. In addition, these differences were observed in male and not female amygdalas, thus showing sex differences.

It is possible that PAE is a risk factor for altered amygdala functioning in early life, which can eventually lead to the development of anxiety and depression in adulthood.

Identifying aberrant factors in key brain regions responsible for regulating stress in the amygdala may eventually elucidate early-life interventions for individual suffering from Fetal alcohol spectrum disorder (FASD).

References


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